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The time course of mandelic and phenylglyoxylic acid excretion in workers exposed to styrene under model conditions

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ABSTRACT Urinary excretion of mandelic and phenylglyoxylic acids by two technicians building glass-reinforced plastic boats has been measured over a 7-day period. Peak excretion of both metabolites occurred several hours after the end of exposure. There was little relationship between urinary mandelic acid concentrations measured at the end of shift and the maximum excretion observed in samples collected after this time. It is suggested that sampling strategies devised to monitor workers exposed to styrene should reflect maximum excretion rates of urinary mandelic acid.

The metabolism of styrene has been extensively studied in both man and animals (Bardodej and Bardodejova, 1970; Leibman and Ortiz, 1970; Leibman, 1975; Ryan and Bend, 1977). The major products of styrene metabolism in man are mandelic and phenylglyoxylic acids (Ohtsuji and Ikeda, 1970; Caperos and Fernandez, 1976; Guillemin and Bauer, 1976; Van Roosmalen and Drummond, 1978). Numerous studies have been reported establishing a correlation between occupational styrene exposure and the excretion of mandelic acid in urine (Slob, 1973; Buchet *et al.*, 1974; Härkönen *et al.*, 1974; Ikeda *et al.*, 1974; Norseth, 1974; Engstrom *et al.*, 1976; Evans *et al.*, 1977). In the design of these studies little attention has been paid to the kinetics of mandelic acid production and excretion. Although Engstrom *et al.* (1976) reported the variability in the half-life of mandelic acid elimination, such pharmacokinetic data have not been used to establish a strategy for biological monitoring of workers exposed to styrene.

In this study we have continuously monitored the mandelic and phenylglyoxylic acid excretion of two technicians involved in an exercise designed to establish optimum ventilation conditions during the manufacture of glass-reinforced plastic boats. This study was also designed to give detailed exposure information and to allow correlation between various forms of static and personal monitoring devices: these data are presented elsewhere.

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The results of the measurements of urinary mandelic and phenylglyoxylic acid excretion are discussed in relation to an optimum strategy for biological monitoring.

Methods

EXPERIMENTAL DESIGN

The two workers monitored over the 7 days of the experiment were involved in a ventilation and environmental monitoring exercise. During this exercise, moulds for a 5-metre boat hull and its superstructure were used under controlled conditions to make a 3-ply glass-reinforced plastic hull and a 2-ply deck moulding during each alternate shift. The hull and deck moulds received a gel-coat by brushing, followed by the required ply of resin/glass fibre mat by hand lay-up (laminating). A general purpose polyester resin dissolved in styrene was used. Ventilation conditions were modified from shift to shift; there were two shifts per day.

Both operatives were provided with accommodation near the exercise and agreed to collect all urine samples over the 7-day period. The urine samples were frozen and stored at $-30^{\circ}C$ as soon after voiding as possible, and were not thawed until analysis.

ANALYSIS OF URINARY METABOLITES

Mandelic and phenylglyoxylic acids were analysed by gas chromatography as their trimethylsilyl derivatives.

Following the addition of phenyllactic acid

(5 μmol) as internal standard, urine samples (1 ml) were adjusted to pH 1 with 2M hydrochloric acid and extracted with diethyl ether (10 ml). The solvent was evaporated and the residue silylated with BSTFA (N,O-bis-(trimethylsilyl)-trifluoroacetamide) (200 μl) and pyridine (100 μl) for 5 min at room temperature. The resulting solution was analysed by gas chromatography. A linear calibration graph was obtained using standard solutions of mandelic acid (up to 20 mmol/l) and phenylglyoxylic acid (up to 10 mmol/l) by expressing the peak area ratio of sample to internal standard against concentration. Quality control samples were analysed with every tenth sample.

For chromatography, a Perkin Elmer F17 gas chromatograph fitted with a flame ionisation detector was used. The glass column (internal diameter, 3 mm; height 2 m) was packed with 3% OV17 on Chromosorb W (80–100 mesh). The injector and detector temperature was set at 175°C and the column temperature at 140°C. The flow rate of nitrogen through the column was 40 ml/min.

The concentration of both metabolites are expressed in terms of creatinine excretion. Urinary creatinine was measured by the Jaffe method using a Technicon AA2 Autoanalyser system.

The exposure of the two technicians to styrene was monitored using personal sampling pumps and charcoal absorption tubes (Simmons and Moss, 1973). The styrene was desorbed from the charcoal with carbon disulphide and estimated by gas chromatography on a Carbowax 20 M column (2 m × 3 mm i.d.) using a flame ionisation detector.

Results

The time-weighted average exposure for both operatives varied from 254 ppm during the first shift (no ventilation) to a minimum of 34 ppm during one of the ventilation exercises. These exposures and the concentrations of mandelic and phenylglyoxylic acids in the urine specimens from each worker are presented in the Figure.

It can be seen that the peak excretion for both metabolites in terms of creatinine concentration occurs several hours after the end of the shift in which exposure took place. There is a parallel between the excretion of mandelic and phenylglyoxylic acids, so that although the ratio between these varies from one urine specimen to another, the overall time course is the same. The time of maximum excretion of both metabolites appears constant for each worker, operative A showing a maximum excretion rate 8 hours after the end of shift on at least the first 3 days, and operative B showing maximum excretion 4 hours after work on four occasions. The

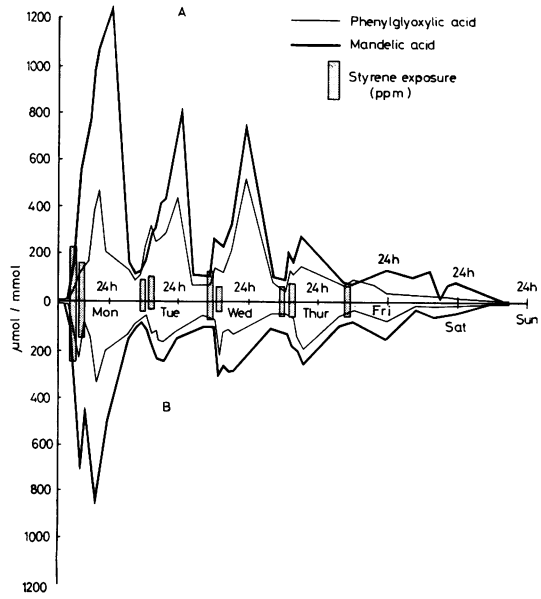

Figure *Urinary excretion of mandelic and phenylglyoxylic acids and its relationship to styrene exposure. The urinary concentration of the two metabolites for technician A are plotted upwards from the horizontal axis and those from technician B, downwards. The values on the ordinate refer both to metabolite excretion (μmol of mandelic acid or phenylglyoxylic acid/mmol of creatinine) and to styrene exposure (time-weighted average; parts per million).*

data also show that there is minimal relationship between peak excretion and the concentration of mandelic acid in urine samples taken early the following morning or directly at the beginning of the next shift.

Discussion

This detailed study of two individuals exposed to styrene under well-documented working conditions enables some conclusions to be drawn about the optimum strategy for collecting urine samples for biological monitoring.

Previous studies have reported correlations between mandelic acid concentration in end-of-shift urine samples and styrene exposure. The correlations reported in these earlier studies have been poor with inter-worker variability surprisingly high. The data from Engstrom *et al.* (1976) show how wide the range of excretion rates is for urinary mandelic acid, the half-life varying from 4·7 to 11·9 hours. It can be seen from these data that the peak excretion of mandelic acid will occur at a wide range of times

after exposure has ended. The data from the two workers presented here show that peak excretion occurs 4 and 8 hours after the end of the exposure. Any sampling procedure dependent on a single urine specimen would not allow for this variability in peak excretion time. One possible method is to collect and pool all urine specimens passed from the end of one shift until the beginning of the next shift. However, work practices vary so that, for certain members of the work force, significant exposure occurs only during the first few hours of the working day. Under these conditions peak excretion may occur before the end of the working period. One approach for dealing with varying exposures during the day would be to collect urine from beginning of work until the end of the waking day. Such a procedure would ensure that the peak excretion period is monitored irrespective of different metabolism and clearance rates and work practices within the working population.

If such a sampling strategy were accepted, the values for mandelic acid excretion for a specific styrene exposure would have to be revised. A preliminary but limited survey using this sampling programme has demonstrated to us that it is acceptable to the work force, and that a better correlation between styrene exposure and mandelic acid excretion is obtainable.

The data presented here for phenylglyoxylic acid excretion show the same time course as mandelic acid excretion, but the ratio between the two metabolites varies considerably from day to day. The sum of the excretion of the two metabolites has been used previously (Ohtsuji and Ikeda, 1970), and the results from our study appear to support such an approach. However, unless urine samples are frozen immediately after collection there is a steady loss of phenylglyoxylic acid due to spontaneous decarboxylation. A further problem is the difficulty in producing a single derivative of phenylglyoxylic acid that is suitable for gas chromatography. Further studies are therefore required to establish whether the measurement of phenylglyoxylic acid, either independently or after reduction to mandelic acid (Guillemin and Bauer, 1976) to give a total excretion for the two metabolites, is a useful procedure.

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